02-14-06

AF

| COMBINED TRANSMITTAL OF APPEAL BRIEF TO THE BOARD OF PATENT APPEALS AND INTERFERENCES & PETITION FOR EXTENSION OF TIME UNDER 37 C.F.R 1.136(a) (Large Entity) | | | Docket No. 675000-353 |
|---|---|---|---|
| In Re Application of: **Lori Ann Wilson** | | | |
| Serial No. **09/751,397** | Filing Date **01/02/2001** | Examiner **Thuy Tran Lien** | Group Art Unit **1761** |
| Invention: **Waxy Wheat Products and Processes for Producing Same** | | | |

OIPE IAP64 FEB 13 2006 PATENT & TRADEMARK OFFICE

TO THE COMMISSIONER FOR PATENTS:

This is a combined Transmittal of Appeal Brief to the Board of Patent Appeals and Interferences and petition under the provisions of 37 C.F.R. 1.136(a) to extend the period for filing an Appeal Brief.

Applicant(s) hereby request(s) an extension of time of (check desired time period):

☐ One Month ☐ Two Months ☒ Three Months ☐ Four Months ☐ Five Months

from: 11/13/2005 until: 2/13/2006

The fee for the Appeal Brief and Extension of Time has been calculated as shown below:

| | |
|---|---|
| Fee for the Appeal Brief | $500.00 |
| Fee for the Extension of Time | $1020.00 |
| **Total Fee for Appeal Brief and Extension of Time:** | **$1520.00** |

The fee for the Appeal Brief and extension of time is to be paid as follows:

☒ A check in the amount of **$1520.00** for the Appeal Brief and extension of time is enclosed.

☐ The Director is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 04-1061.

☐ Please charge the deposit account in the amount of **1520.00**.
☒ Any additional filing fees required under 37 CFR 1.16.
☐ Any patent application processing fees under 37 CFR 1.17.

☒ If an additional extension of time is required, please consider this a petition therefore and charge any additional fees which may be required to Deposit Account No. 04-1061.

Randall L. Shoemaker, Reg. No. 43,118
Dickinson Wright, PLLC
38525 Woodward Ave, Ste. 2000
Bloomfield Hills, Michigan 48304-2970
(248) 433-7200

**Certificate of Mailing Under 37 C.F.R. 1.10**

I hereby certify that this correspondence is being deposited with the United States Postal Service bearing Express Mail Label No: EV 857774305 US in an envelope addressed to: Mail Stop: Appeal Brief – Patents; Commissioner for Patents, PO BOX 1450, Alexandria, Virginia 22313-1450,

on 2·13·2006

Rainie L. Mills

02/15/2006 CCHAU1 00000010 09751397
02 FC:1253 1020.00 OP

EV857774305US

Attorney Docket No: 675000-353

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | | |
|---|---|---|---|---|
| **Applicant** | : | **Lori Ann Wilson** | **: Group:** | **1761** |
| **Serial No.** | : | **09/751,397** | **: Examiner:** | **Thuy Tran Lien** |
| **Filed** | : | **01/02/2001** | **: Confirmation No.** | **7520** |
| **Title** | : | **WAXY WHEAT PRODUCTS AND PROCESSES FOR PRODUCING SAME** | | |

## BRIEF ON APPEAL

Mail Stop Appeal Brief – Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

Subsequent to the filing of the Notice of Appeal dated September 13, 2005 and acknowledged by the OIPE, Applicants now submit a brief in support of the appeal in response to the Final Rejection set forth in the Office Action dated June 15, 2005. A single copy of this Appeal Brief is being submitted in accordance with 37 C.F.R. §41.37 and this Appeal Brief is accompanied by the required fee of $500.00 under §41.20(b). In addition, Applicants have enclosed a request for a three month extension of time and a check in the amount of $1,520.00.

The Patent Office is authorized to charge or refund any fee deficiency or excess to Deposit Account No. 04-1061.

02/15/2006 CCHAU1 00000010 09751397
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PATENT & TRADEMARK OFFICE

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## I. Real Party in Interest

The real parties in interest are The United States of America as represented by the Secretary of Agriculture as recorded on reel/frame 012662/0902 recorded on May 6, 2002 and Kellogg Company as recorded on reel/frame 0132001/0288 recorded on August 19, 2002. This appeal brief is being filed on authorization from both real parties in interest.

## II. Related appeals and interferences

There are no related appeals or interferences.

## III. Status of claims

Claims 1-3, 6 and 8-51 remain in the application, claims 4, 5 and 7 have been cancelled. Claims 13-33 and 36-51 have been withdrawn from consideration. Claims 1-3, 8-12, 34 and 35 have been finally rejected and are the subject of this appeal. Claim 6 has not been rejected by the Examiner, thus it is not part of this appeal. Applicants assume that claim 6 is allowable if rewritten in independent form to incorporate all the limitations of independent claim 1 and claim 6.

## IV. Status of amendments

All amendments have been entered and are reflected in the claims in the claims appendix.

## V. Summary of claimed subject matter

The present invention is directed to a cooked buoyant whole grain waxy wheat product. For a general description see the specification at the following: page 1, lines 3-10; page 5, lines 21-26; page 7, lines 16-19; page 10, lines 14-18; page 11, lines 15-24; and page 13, lines 1-4. Preferably the whole grain waxy wheat product comprises less than about 10% amylose starch. See page 1, lines 3-10; page 8, lines 20-21; and page 9, lines 1-6. The waxy wheat preferably has less than 20% protein by weight and more preferably are less than or equal to 14% protein by weight. See page 1, lines 3-10 and page 9, lines 1-6. The cooked buoyant whole grain waxy wheat is gelatinized throughout. See pages 5, lines 24-28; page 7, lines 20-24; and page 11, lines 20-24. The whole grain waxy wheat product is storage stable in the absence of additives that inhibit rancidity preferably for at least six months and more preferably for at least twelve months. See page 1, lines 3-10; page 7, lines 8-13; page 10, line 19 through page 11, line 15; and page 16, line 20 through page 17, line 11. The product is either the whole grain kernels or a ground whole grain kernel. See pages 7, lines 20-24; page 8, lines 1-7; and page 11, lines 20-24. Most preferred is a waxy wheat that is a Wx-D1 null, Wx-A1 null, and Wx-B1 null allele. See page 9, line 24 through page 10, line 8. Preferably once prepared the waxy wheat product is further processed to include an edible coating. See page 15, line 9 through page 16, line 3; and page 16, lines 12-19. Whether coated or uncoated the waxy wheat product can then be processed into a variety of food forms including cereals, bars, granola, biscuits, crackers, breads, muffins and other food forms. See page 14, line 3 through page 15 line 11. The waxy wheat product is preferably produced by a process wherein the waxy wheat is heated for a period of time of from about 5 to about 15 minutes at a temperature of from about 94°C to about 110°C with moisture.

The heated waxy wheat can then be tempered for a period of time of from about 1 to about 2 hours, but this step is optional. The waxy wheat is then gelatinized throughout preferably by cooking for about 45 to about 90 minutes at a temperature of from 93°C to about 177°C to gelatinize the waxy wheat throughout. The gelatinized waxy wheat is then cooled and dried preferably to a moisture content from about 10 to about 16%. See examples 1-3, figures 1, 2, and page 11, line 20 through page 14, line 2.

## VI. Grounds of rejection to be reviewed on appeal

The first ground of rejection is whether claims 34-35 are indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention under 35 U.S.C. §112, second paragraph. Second ground of review is whether claims 1-3, 8-12, and 34-35 are properly rejected under 35 U.S.C. §103(a) as being unpatentable over Alderman US Pat No. 2,526,792 in view of Nakamura et al. "reduction of waxy amylose free wheats" and the book "Wheat Chemistry and Technology".

## VII. Argument

### A. Rejection of claims 1-3 and 8-12 under 35 U.S.C. § 103(a) based on Alderman (US 2,526,792) in view of Nakamura et al. and the book "Wheat Chemistry and Technology".

The Examiner rejected claims 1-3 and 8-12 under 35 U.S.C. §103(a) based on Alderman in view of Nakamura et al. in the book "Wheat Chemistry and Technology". The Examiner relies on Alderman as disclosing a cooked-puffed waxy cereal food formed into ready-to-eat cereal foods of the breakfast type. The Examiner relies on Nakamura et al. as disclosing the production of waxy wheat grains. The Examiner relies on the book as disclosing the protein contents of soft and hard wheats. Utilizing the disclosure of Nakamura et al. the Examiner states it would be obvious to use waxy wheat to produce the waxy cooked cereal grains disclosed by Alderman. The Examiner assumes that when waxy wheat is used it would be obvious that the grains would have the claimed allele and amylose content of the present application. The Examiner furthermore states that the book discloses that the common range of protein is from 9-15% and thus states it would be obvious to utilize a waxy wheat having that protein level. Still referring to the Alderman disclosure the Examiner states "Since the cereal product is puffed and it is a ready-to-eat cereal, the product is buoyant because cereal floats in liquid. The Alderman product is dried; it is made of cereal grain and it is the same type of product as claimed. Thus, it is obvious the product has the same storage stability as claimed."

Rejection of a claim under 35 U.S.C. §103 requires that the Examiner point to some teaching, suggestion, or motivation found within the references themselves that would lead one of ordinary skill in the art to combine the references and having combined the references to make the claimed invention obvious in light of a teaching, suggestion or motivation found within the

references themselves. Absent the Examiner's ability to point to such a specific teaching, suggestion, or motivation the rejection of the claims under 35 U.S.C. §103 based on a combination of references is improper and must be withdrawn. Claim 1 is in independent form and states "cooked, buoyant whole grain waxy wheat comprising no more than about 10% amylose starch, and less than 20% by weight protein characterized by being gelatinized throughout and storage stable in the absence of additives that inhibit development of rancidity for at least six months." The product disclosed in Alderman and the process utilized by Alderman do not produce the product as claimed in claim 1 of the present application. Specifically, the Alderman process does not result in a waxy grain, and certainly not a waxy wheat that is storage stable for greater than six months in the absence of additives that inhibit rancidity. In addition, the product disclosed in Alderman and produced using the process of Alderman is not a whole grain waxy product. Accompanying this Appeal Brief is a declaration under 37 CFR §1.132 which includes submission of supporting data. The declaration is signed by and the data was generated under the direction of Lori Wilson, one of the inventors of the present invention. The declaration presents evidence directly comparing a waxy wheat that is processed according to Alderman versus one processed according to the present application. The declaration provides data that clearly indicates that the two processes produce entirely different products and that the product claimed in the present application is different and certainly not obvious in view of the product and processes disclosed in Alderman and the other cited references. The data specifically disclose that waxy wheat taken through the process of Alderman does not result in a whole grain, does not result in complete gelatinization, and that the product produced is not storage stable in the absence of additives that inhibit the development of rancidity.

The declarant has over twenty years of experience in the area of research and development in quality control in cereals and cereal processing at the Kellogg Company. The declarant has a BS in food science and human nutrition from Michigan State University. To demonstrate that the whole grain waxy wheat of the present invention is different from and not obvious based on the disclosure of Alderman, Nakamura et al. and the book the declarant took two portions of a whole grain waxy wheat from the same grain lot and origin. One portion was treated per example 5 of Alderman, which is the only example wherein he uses a whole grain in his process, and the other portion was treated as per the present application. As noted in the declaration and is known by ordinary skill in the art in the industry "whole grain" as utilized in the present claims is defined as intact kernels of grain or fractions thereof that contain all three parts of the whole grain, namely the outer bran, the endosperm, and the germ.

It is noted in paragraph 6 of the declaration to test the Alderman process 15 pounds of the whole grain waxy wheat were combined with 1.31 pounds of sugar, 0.44 pounds of salt, and 5 pounds of water. The mixture was cooked at 15 pounds per square inch of steam for one hour and forty minutes in a rotary cooker at 2.3 rpms. The cooked waxy wheat was then dried at 250°F for approximately 40-50 minutes to a final moisture of 16.1%. The cooked waxy wheat was then tempered per the Alderman procedure for 24 hours. The cooked and tempered waxy wheat was then run through flaking rollers. Finally, the milled, cooked waxy wheat was toasted per example 5 of Alderman. The resulting product was then tested for a variety of parameters related to claim 1 including the amount of whole grain, the degree of gelatinization, and storage stability.

The other portion of whole grain waxy wheat was treated as described in paragraph 7 of the declaration in accordance with the present application. In a first step 23 pounds of the whole grain waxy wheat was placed in a rotary cooker and steamed at 15 pounds per square inch for 15 minutes at 2.3 rpms. As described in the present application, the steaming process inactivates the lipases which are believed to make cooked whole grain waxy wheat unstable and subject to rancidity. The steamed whole grain waxy wheat was then bumped by passing it through rollers set at 300 mm. The bumping of the uncooked waxy wheat causes small fissures in the bran layer but does not remove any of the bran layer. Then 15 pounds of the bumped waxy wheat was mixed with a slurry of 1.31 pounds of sugar, 0.44 pounds of salt, and 3.0 pounds of water. The fissures allow the slurry to penetrate the whole grain during the cooking process. The mixture was cooked in a rotary cooker 15 pounds per square inch, 2.3 rpms for 45 minutes during the cooking process the whole grain waxy wheat will tend to become like glue and holds the outer bran layer onto the whole grain. The cooked whole grain waxy wheat was then dried at 250°F for approximately 20 minutes to a final moisture of 16.4%. Next the warm, dried whole grain waxy wheat was roller milled while at a temperature of from 100 - 120°F and then cooled and tempered for 24 hours. Roller milling the cooked whole grain waxy wheat while it is warm ensures that the starches are at a temperature above their glass transition state so that they are plyable and can be formed without shattering. Finally, the tempered whole grain waxy wheat was toasted as noted for Alderman. The resulting product was then tested for a variety of parameters related to claim 1, including the amount of whole grain, the degree of gelatinization, and storage stability.

One of the key limitations in claim 1 of the present invention is that the wheat be "a whole grain waxy wheat". As noted above, whole grain has a very specific definition in the industry and is known to those of ordinary skill in the art to require that all three portions of the wheat kernel be present, namely, the outer bran, the endosperm, and the germ. Soluble and insoluble fiber of waxy grains is found in the outer layer of the grain, thus measuring the level of insoluble and total fiber in a product is commonly used in the industry to determine whether a grain is a whole grain or less than whole grain. Such measures of the intactness of the whole grain is common and well known to those of ordinary skill in the art. Because both wheats were taken from the same source they should produce the same level of insoluble in total fiber provided that they both represent whole grain waxy wheat. The product produced according to the method of Alderman had an insoluble fiber level of 6.29 weight percent and a total fiber level of 9.99 weight percent. By way of contrast, the product as claimed in claim 1 had an insoluble fiber level of 10.14 weight percent and a total fiber level of 13.56 weight percent. This data is presented in paragraph 8 of the declaration. Thus, the product prepared according to the present invention had an insoluble fiber level that was 161% of the value found in the grain according to Alderman and a total fiber level that was 135% of the total fiber found within Alderman. Clearly, these results demonstrate that the product of the present invention is a whole grain whereas Alderman has lost a portion of the outer layer which is where the fiber is found.

Claim 1 further requires that the whole grain waxy wheat be gelatinized throughout. As known to those of ordinary skill in the art the degree of gelatinization can be measured in a number of ways including by measuring water solubility, alkali solubility, and by rapid viscosity analysis. As known to those of ordinary skill in the art the higher the percentage of water

solubility or alkali solubility the greater the degree of gelatinization. The water solubility is measured in terms of grams per 100 grams in water. The Alderman product had a value of 18% while the product prepared according to the present invention had a value of 23%. The alkali solubility of the Alderman product was 50% while the product of the present invention had a value of 66%. The viscosity measurements were taken over time as the temperature of the solution is varied and can be seen in the attached figure of the declaration throughout the entire range of time and temperature the Alderman product had a much higher viscosity which is a clear indication that it is less gelatinized in the product as claimed in claim 1 of the present invention. The higher degree of water solubility and alkali solubility of the product prepared according to the present invention also indicates that the product was more thoroughly gelatinized than that of Alderman. These measures for gelatinization are described and discussed in paragraphs 9 of the declaration. Those of ordinary skill in the art know how these measurements are performed and that they are commonly used to evaluate gelatinization of grains.

Finally, claim 1 requires that the product be stable in the absence of additives that inhibit rancidity for at least six months. The data in the specification shows that this is easily achievable with the product of the present invention and in fact stability beyond twelve months is also easy to achieve. The stability of the Alderman product and the product prepared according to the present invention were tested using two protocols and by measuring the head space hexanal use of this measurement is described in the specification page 10, lines 4-13 and page 21, line 30 through page 23. Measurement of hexanal levels in headspace are commonly used in the industry to detect rancidity in cereal products. The hexanal levels in the headspace were

measured after storage from four months at 70°F and 35% relative humidity which represents ambient storage conditions for cereal products for four months. In a second protocol some of the samples were moved to conditions of 100°F and 70° relative humidity for either two or eight additional weeks after the four month storage period. Storage under these high temperature and humidity conditions is used in the industry to simulate longer term storage at ambient conditions in an accelerated fashion. The results were presented in paragraph 10 of the declaration. The results dramatically showed that the headspace hexanal level in the Alderman product was higher at all points and conditions measured compared to the product prepared in accordance with the present invention. In fact, after the first two weeks of accelerated conditions the Alderman product had a hexanal headspace level that was 358% of that measured at four months. The product prepared according to the present invention only had an increase of 193% of the four month measure and was significantly lower than that found in the Alderman product. By the end of eight weeks of accelerated conditions the Alderman product had a hexanal headspace level of 698% of the four month measure and was clearly rancid. In the industry headspace hexanal levels greater than 5 ppm are considered to represent clear rancidity. By way of contrast, the present invention product was still well below the Alderman product and well below the rancidity threshold.

In summary, the declaration shows by a number of different measures that the product of the present invention is clearly different from and not obvious in view of the disclosure of Alderman, Nakamura et al. and the book. Furthermore, the results clearly demonstrate that even if it were obvious to substitute waxy wheat of Nakamura et al. in the Alderman process following that process would not produce a product as claimed in claim 1 of the present

invention. This is so because that is precisely what was tested in the declaration, namely taking a waxy whole wheat grain and following the Alderman process and comparing that to one according to the present invention. The Examiner tries to state that there was not sufficient data from which one could conclude that this was the case because the experiments were only performed once. While it may be true that in a isolated measure a single repetition could be insufficient here we have a situation where numerous parameters were measured all of which clearly demonstrate that three of the key limitations found in claim 1 are not present in a product produced according to the Alderman process. Therefore, the rejection of claim 1 and the claims which depend therefrom under 35 U.S.C.§103 based on Alderman, Nakamura et al. and the book is improper and should be withdrawn.

**B. Rejection of claims 34 and 35 under 35 U.S.C. § 103(a) based on Alderman (US 2,526,792) in view of Nakamura et al. and the book "Wheat Chemistry and Technology".**

The Examiner rejected claims 34 and 35 under 35 U.S.C.§103(a) based on Alderman in view of Nakamura et al. and the book. As discussed above the attached declaration clearly shows that products produced according to the Alderman process are unlike that claimed in the present invention. To summarize, the product according to the present invention is a whole grain product whereas that produced according to the Alderman process is not whole grain. The product of the present invention is storage stable whereas that of Alderman is not. Finally, the product of the present invention is gelatinized throughout whereas the product of Alderman is not fully gelatinized. In summary, the product claimed in claims 34 and 35 is not obvious based on Alderman in view of Nakamura and the book.

**C. Rejection of claims 34 and 35 under 35 U.S.C. § 112, second paragraph as indefinite.**

The Examiner has declared that claims 34 and 35 are indefinite because they are based on claims that have been withdrawn. Claims 34 and 35 are a product that do refer to withdrawn claims, however, they claim a product that is definite, namely a cooked buoyant whole grain waxy wheat produced according to the process of either claim 13 or 32. Thus, Applicants submit that the claims are not indefinite and that Examination of the claims should proceed with this rejection being removed.

**Respectfully submitted,**

**DICKINSON WRIGHT, PLLC**

**February 13, 2006**
Date

**Randy Shoemaker, Reg. No. 43,118**
Dickinson Wright, PLLC
38525 Woodward Avenue, Suite 2000
Bloomfield Hills, MI 48304-2970
(248) 433-7383

## CERTIFICATE OF EXPRESS MAILING

I hereby certify that the attached Appeal Brief for application serial number 09/751,397 filed January 2, 2001 is being deposited with the United States Postal Service as Express Mail EV 857774305 US in an envelope addressed to Mail Stop Appeal Brief – Patents, Commissioner of Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450, on this **February 13, 2006**.

Rainie Mills

**Rainie Mills**

## VIII. Claims appendix

1. (Previously presented) Cooked, buoyant whole grain waxy wheat comprising no more that about 10% amylose starch, and less than 20% by weight protein characterized by being gelatinized throughout and storage stable in the absence of additives that inhibit development of rancidity for at least 6 months.

2. (Original) Waxy wheat of claim 1, comprising a protein content of no more than about 14% by weight.

3. (Previously presented) Waxy wheat of claim 1 in the form of integral whole grain kernels or ground whole grain kernels.

Claims 4-5 (Cancelled)

6. (Previously presented) Waxy wheat of claim 1, wherein the waxy wheat comprises a Wx-D1 null, Wx-Al null and Wx-B 1 null allele.

7. (Cancelled)

8. (Previously presented) Waxy wheat of claim 1 wherein said cooked buoyant whole grain waxy wheat is storage stable for at least about 12 months.

9. (Original) Waxy wheat of claim 1, further comprising an edible coating.

10. (Original) Waxy wheat of claim 9, wherein the coating is selected from the group consisting of sucrose, dextrose, rice syrup, carnauba wax, polymeric fructose, corn syrup solids and oil.

11. (Previously presented) Edible composition comprising the cooked, buoyant whole grain waxy wheat of claim 1.

12. (Previously presented) Edible composition of claim 11 selected from the group consisting of ready to eat cereals, muesli, granola grain clusters, snack bars, biscuits, crackers, bread, cakes, muffins and pie crusts.

13. (Withdrawn) Process for preparing a cooked, buoyant, waxy wheat, comprising:

(a) heating a waxy wheat having no more than about 10% amylose for about 5 to about 15 minutes at about 200°F (94°C) to 230°F (110°C) with moisture,

(b) gelatinizing the heated waxy wheat throughout, and

(c) cooling and drying the gelatinized waxy wheat,

wherein said wholegrain waxy wheat product is storage stable for at least about six months in the absence of additives that inhibit development of rancidity.

14. (Withdrawn) Process of claim 13, wherein said waxy wheat is heated for about 5 to about 10 minutes with steam and then tempering the waxy wheat for about 1 hour to about 2 hour.

15. (Withdrawn) Process of claim 14, wherein said tempering is about 1 hour at ambient temperature.

16. (Withdrawn) Process of claim 14, wherein said tempering is for about 1 hour at about 160°F (71°C) to about 200°F (93°C).

17. (Withdrawn) Process of claim 13, wherein the waxy wheat in step (b) is heated for about 45 minutes to about 90 minutes at 200°F (93°C) to about 350°F (177°C) to gelatinize the waxy wheat.

18. (Withdrawn) Process of claim 13, wherein the waxy wheat in step (b) is heated for about 1 hour at about 260°F (127°C).

19. (Withdrawn) Process of claim 13, further comprising separating the cooled waxy wheat in step (c) into separate kernels prior to drying.

20. (Withdrawn) Process of claim 19, further comprising toasting the separated dried kernels.

21. (Withdrawn) Process of claim 19, further comprising drying the separated kernels to a moisture content of 10 to 16% then heating the kernels to about 380°F (193°C) to about 700°F (371°C) for 15 to 25 seconds.

22. (Withdrawn) Process of claim 13, wherein flavorings are added to the waxy wheat prior to, during or after gelatinization.

23. (Withdrawn) Process of claim 13, wherein the waxy wheat comprises a protein content of about less than 14% by weight.

24. (Withdrawn) Process of claim 13, wherein the waxy wheat comprises Wx-D1 null, Wx-Al or Wx-B1 null allele.

25. (Withdrawn) Process of claim 13, further comprising kneading the gelatinized and cooled waxy wheat of step (c) under low shear to form a dough.

26. (Withdrawn) Process of claim 25, further comprising shaping and drying the dough to a moisture content of 10 to 16%.

27. (Withdrawn) Process of claim 26, wherein further comprising toasting or puffing the shaped dough.

28. (Withdrawn) Process of claim 27, wherein the dried dough is puffed by heating the shaped dough to about 380°F (193°C) to about 700°F (371°C).

29. (Withdrawn) Process of claim 13, wherein the waxy wheat comprises a protein content of less than 14% by weight of the grain.

30. (Withdrawn) Process of claim 13, comprising

(a) heating the waxy wheat for 5 to 7 minutes at about 17 psi, then

(b) tempering the heated waxy wheat for about 1 hour, then

(c) cooking the tempered waxy wheat for about 1 hour to about 280°F (138°C) to gelatinize the waxy wheat, then

(d) kneading the gelatinized waxy wheat under low shear to form a dough, then

(e) shaping the dough and

(f) then drying the dough to a moisture content of about 10% to 16%.

31. (Withdrawn) Process of claim 13, further comprising puffing or toasting the dried waxy wheat of step (c).

32. (Withdrawn) Process for preparing a cooked, buoyant, waxy wheat, comprising

(a) heating a waxy wheat for about 5 to about 10 minutes with steam,

(b) then tempering the heated waxy wheat for about 1 to about 2 hours,

(c) cooking the tempered waxy wheat for about 45 minutes to about 90 minutes at 200°F (93°C) to about 350°F (177°C) to gelatinize the wholegrain waxy wheat throughout,

(d) cooling and separating the gelatinized wholegrain waxy wheat, and then

(e) drying the separated wholegrain waxy wheat to a moisture content of about 10% to 16%.

33. (Withdrawn) Process of claim 32, further comprising puffing or toasting the wholegrain waxy wheat of step (e).

34. (Previously presented) Cooked, buoyant, wholegrain waxy wheat produced by the process of claim 13.

35. (Previously presented) Cooked, buoyant, wholegrain waxy wheat produced by the process of claim 32.

36. (Withdrawn) Process of claim 13, wherein the waxy wheat of step (a) is milled after heating and prior to gelatinizing to produce a ground meal.

37. (Withdrawn) Process of claim 36, further comprising shaping the gelatinized ground meal and drying to a moisture content of about 10% to 16%.

38. (Withdrawn) Process of claim 36, wherein the ground meal is gelatinized in a a rotary cooker or a cooker-extruder having a die face.

39. (Withdrawn) Process of claim 36, further comprising extruding the gelatinized ground meal and forming the extruded ground meal into a product of a desired shape..

40. (Withdrawn) Process of claim 39, further comprising toasting or puffing said shaped product.

41. (Withdrawn) Process of claim 39, wherein the shaped product is puffed by heating to about 380°F (193°C) to about 700°F (371°C).

42. (Withdrawn) Process of claim 36, wherein the ground meal is gelatinized in a cooker-extruder and directly expanded.

43. (Withdrawn) Process of claim 13, further comprising milling the gelatinized barley of step (c) to produce a ground meal.

44. (Withdrawn) Process of claim 43, wherein said ground meal is formed into a product having a desired shape.

45. (Withdrawn) Process of claim 44, wherein the shaped product is a flake, shred, puff, nugget, strip or chip.

46. (Withdrawn) Process of claim 44, wherein the shaped product is toasted or puffed.

47. (Withdrawn) Process of claim 44, wherein the shaped product is dried to a moisture content of about 10% to 16%.

48. (Withdrawn) Process of claim 44, further comprising toasting or puffing the dried shaped product.

49. (Withdrawn) Process of claim 13, wherein the waxy wheat in step (c) is bumped, flaked, puffed or toasted.

50. (Withdrawn) Process of claim 13, wherein the waxy wheat is gelatinized in a cooker-extruder having a die face and is directly expanded at the die face.

51. (Withdrawn) Process of claim 50, wherein the directly expanded gelatinized waxy wheat is toasted.

## IX. Evidence appendix

Attached are copies of the references relied on by the Examiner in the final rejection, namely: Alderman (US 2,526,792), Nakamura et al., and the excerpt from "Wheat Chemistry and Technology". In addition, a copy of the declaration under 37 C.F.R. §1.132 submitted with the response to the office action of September 23, 2004 is included. The Examiner admitted this declaration in the final rejection mailed Jaune 15, 2005.

## X. Related proceedings index

None.

BLOOMFIELD 675000-353 745121v1

Attorney Docket # 67,500-353

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | |
|---|---|
| In re application of: | Wilson, et al. |
| Serial No.: | 09/751,397 |
| Group Art Unit: | 1761 |
| Filed: | January 2, 2001 |
| Examiner: | Tran, Lien Thuy |
| For: | **WAXY WHEAT PRODUCTS AND PROCESSES FOR PRODUCING SAME** |

**DECLARATION UNDER 37 C.F.R. § 1.132**
**SUBMISSION OF SUPPORTING DATA**

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, Lori Ann Wilson, hereby state that:

1. I am one of the inventors of the present application and a person of ordinary skill in the art.

2. I received a B.S. in Food Science and Human Nutrition from Michigan State University in 1980. I began working in the Research and Development area at the Kellogg Company in 1980. For the first 16 years I worked in cereals and cereal processing. For the last 7.5 years I have worked in the Quality area of cereals and cereal processing for the Kellogg Company.

3. This Declaration provides evidence that our (i.e., the Applicants') invention is not obvious to a person of ordinary in the art over Alderman (United States Patent No. 2,526,792) in view of Nakamura et al. (Production of waxy (amylose free) wheats, in Mol. Gen. Genet. 248: 253-259 (1995)) and the book Wheat Chemistry and Technology, edited by Y. Pomeranz, 3rd Ed. 1988, pp 10 and 16-17. I am aware of, have read, and understand the cited references.

4. To demonstrate that the whole grain waxy wheat of the present application is different from and not obvious based on Alderman I took two portions of whole grain waxy wheat from the same grain lot and origin. One portion was treated per Example 5 of Alderman as described below, the only example wherein he uses a whole grain in his disclosed process. The other portion was treated as per the present application as described below.

5. In the industry whole grain is defined as intact kernels or fractions thereof that contain the three parts of the whole grain, namely the outer bran, the endosperm, and the germ.

6. To test the Alderman process 15 pounds of the whole grain waxy wheat were combined with 1.31 pounds of sugar, 0.44 pounds of salt, and 5 pounds of water. The mixture was cooked at 15 pounds per square inch of steam for 1 hour and 40 minutes in a rotary cooker at 2.3 rpms. The cooked waxy wheat was then dried at 250° F for approximately 40 to 50 minutes to a moisture of 16.1%. The cooked waxy wheat was then tempered per the Alderman procedure for 24 hours. The cooked and tempered waxy wheat was then run through flaking rollers. Finally, the milled, cooked, waxy wheat was toasted as per Example 5 of Alderman. The resulting product was then tested for a variety parameters related to claim 1 included the amount of whole grain, degree of gelatinization, and storage stability.

7. The other portion of whole grain waxy wheat was treated per the present application. In a first step 23 pounds of the whole grain waxy wheat was placed in a rotary cooker and steamed at 15 pounds per square inch for 15 minutes at 2.3 rpms. As described in the present application, this steaming process serves to inactivate the lipases, which are believed to make cooked whole grain waxy wheat unstable and subject to rancidity. This steaming process also makes the bran layer pliable. The steamed whole grain waxy wheat was then bumped by passing it through rollers set at 300 mm. The bumping of the uncooked waxy wheat creates small fissures in the bran layer, but does not remove any of it. Then 15 pounds of the bumped waxy wheat was mixed with a slurry of 1.31 pounds of sugar, 0.44 pounds of salt, and 3.0 pounds of water. The fissures allow the slurry to penetrate the whole grain during the cooking process. The mixture was cooked in a rotary cooker at 15 pounds per square inch, 2.3 rpms, for 45

minutes. During the cooking process according to the present invention the starches in the whole grain waxy wheat tend to become glue like and hold the outer bran layer onto the whole grain. The cooked whole grain waxy wheat was then dried at 250° F for approximately 20 minutes to a moisture of 16.4%. Next the warm, dried whole grain waxy wheat was roller milled while at a temperature of from 100 to 120° F. The milled grain was cooled and then tempered for 24 hours. In the process of the present invention roller milling the cooked whole grain waxy wheat when it is warm ensures that the starches are above the glass transition state so they are pliable and can be formed without shattering. Finally, the tempered whole grain waxy wheat was toasted as above. The resulting product was then tested for a variety parameters related to claim 1 included the amount of whole grain, degree of gelatinization, and storage stability.

8. The amount of insoluble fiber and the amount of total fiber in each product was measured and compared. The fiber is found in the outer layer of the grain and its level goes down as a grain is converted from a whole grain to a processed grain. Thus, this is a measure of whether each is a "whole grain waxy wheat" as required by claim 1. The product of Alderman had an insoluble fiber level of 6.29 weight % and a total fiber level of 9.99 weight %. By way of contrast, the product as claimed in claim 1 had an insoluble fiber level of 10.14 weight % and a total fiber level of 13.56 weight %. Clearly, the Alderman product is not a whole grain as claimed in claim 1 since it has a lower insoluble and total fiber level compared to the product as claimed in claim 1.

9. The degree of gelatinization can be measured in a number of ways including by measuring water solubility, alkali solubility, and by rapid viscosity analysis. The higher the percentage of water solubility or alkali solubility the greater the degree of gelatinization. The water solubility is in terms of grams per 100 grams in water. The Alderman product had a value of 18 % while the product of the present invention had a value of 23%. The alkali solubility of the Alderman product was 50% while the product of the present invention had a value of 66%. Clearly, by either measurement the Alderman product is not gelatinized throughout as required by claim 1 and as shown in the present invention. The results from the rapid viscosity analysis are attached as Exhibit 1. The viscosity is measured over time as the temperature of the solution is varied. One can see that throughout the entire range of time and temperature the

Alderman product has a much higher viscosity indicating that it is less gelatinized than the product as claimed in claim 1 of the present invention. All of these measures establish that the Alderman product is not gelatinized throughout compared to the product of the present invention and as required by claim 1.

10. The stability of the Alderman product and the product of the present invention were tested using two protocols and measuring the headspace hexanal. This is described in the present specification on page 10, lines 4 – 13 and page 21, line 30 through page 23. In a first protocol the hexanal levels in the headspace were measured after storage for 4 months at a temperature of 70° F and 35% relative humidity representing ambient conditions for 4 months. In a second protocol some of the samples from the first protocol were moved to conditions of 100° F and 70% relative humidity for either 2 or 8 more weeks. Storage under these conditions is used in the industry to simulate longer term storage at ambient conditions in an accelerated fashion. The protocols are well recognized in the food industry. The results are provided in Table 1 below:

TABLE 1

| Condition of storage | Alderman product, headspace hexanal in ppm | Present invention product, headspace hexanal in ppm |
|---|---|---|
| 4 months, 70° F 35% relative humidity | 1.0 | 0.66 |
| 2 weeks, 100° F, 70% relative humidity | 3.58 | 1.28 |
| 8 weeks, 100° F, 70% relative humidity | 6.98 | 3.49 |

The results demonstrate that the Alderman product is not storage stable. After the initial storage of 4 months the Alderman product has significantly more hexanal than the present invention. After the first 2 weeks of accelerated conditions the Alderman product has a hexanal level that is 358% of the measure at 4 months while the present invention is only at 193% of the 4 month measurement. By the end of the 8 weeks of accelerated conditions the Alderman product has a hexanal level of 698% of the 4 month

level and is clearly rancid. Headspace hexanal levels greater than 5 ppm are considered in the industry to represent clear rancidity. By way of contrast, the present invention product is still well below the Alderman product and well below any rancidity threshold.

11. In summary, even if one of ordinary skill were to follow the teachings of the Alderman process using a waxy wheat after reading Nakamura et al. and the book Wheat Chemistry and Technology one could not produce a "Cooked, buoyant, whole grain waxy wheat comprising no more than about 10% amylose starch, and less than 20% by weight protein characterized by being gelatinized throughout and storage stable in the absence of additives that inhibit development of rancidity for at least 6 months." as required by claim 1 of the present application. Alderman alone or in combination with the other cited references does not make the present whole grain waxy wheat product obvious.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true; and further that these statements were made with the knowledge that willful and false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or a patent issued thereon.

Lori Ann Wilson

Lori Ann Wilson

03/23/2005

Execution Date

Citizenship: United States
Residence: 13606 C Avenue East
Hickory Corners, Michigan
USA

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PATENT & TRADEMARK OFFICE

Attorney Docket # 67,500-353

# CERTIFICATE OF MAILING BY "EXPRESS MAIL"

I hereby certify that the enclosed **Declaration Under 37 C.F.R. § 1.132** is being deposited with the United States Postal Service as Express Mail, postage prepaid, in an envelope as "Express Mail Post Office to Addressee", **Mailing Label No. ########US** and addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on **March 23, 2005.**

Rainie Mills

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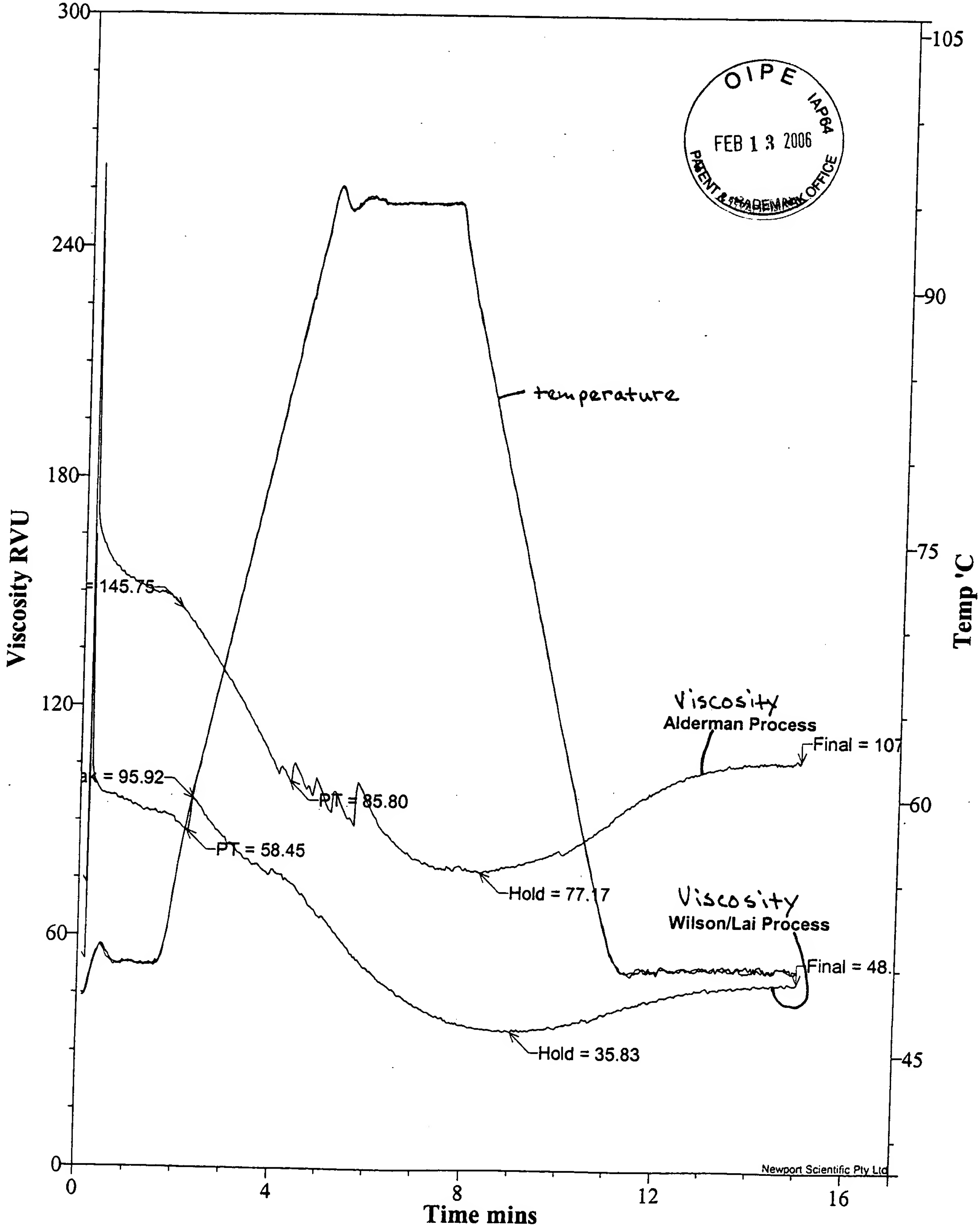
# RVA Comparison of Alderman and Wilson/Lai Processes



# UNITED STATES PATENT OFFICE

2,526,792

## PUFFED WAXY CEREAL FOOD AND PROCESS OF MAKING SAME

Mattison Wells Alderman, Battle Creek, Mich., assignor to General Foods Corporation, New York, N. Y., a corporation of Delaware

No Drawing. Application September 30, 1946, Serial No. 700,255

9 Claims. (Cl. 99—81)

This invention relates to puffed ready-to-eat cereal foods of the breakfast cereal type.

Practically all of the common cereal grains can be puffed successfully by the method known as "gun-puffing" wherein the kernels are subjected to steam under high pressure to convert their moisture content into entrapped steam. The pressure is then released suddenly whereupon the cell walls burst and the kernels expand to as much as eight times their original volume. Gun-puffed cereals of various kinds have been on the market for a long time and are well known. Such products characteristically have substantially smooth outer surfaces and spongy or pithy compressible interiors. As a rule they are not toasted since toasting does not take place during puffing but requires a subsequent treatment.

In distinction to gun-puffing, the method commonly known as "oven-puffing" simply subjects the cooked cereal to relatively high temperatures in the range of 300–600° F. without the application and sudden release of pressure that are characteristic of gun-puffing. Oven-puffing thus produces a product which differs markedly from that obtained with gun-puffing in that it is toasted, frangible and crisp and sufficiently impervious to moisture absorption that when placed in milk or cream it retains its crisp, friable character for a reasonable period. These characteristics of oven-puffed products are very desirable in breakfast cereals, but the general use of this method of puffing has not been practicable heretofore because of the limited amount of puffing produced thereby.

Many expedients have been used in an attempt to overcome the above mentioned disadvantage of oven-puffing. For example, moderate compression or flattening of rice grains between spaced rolls prior to the oven treatment increases the extent of puffing sufficiently to produce an acceptable commercial product. However, this additional and hence undesirable operation does not produce the desired degree of puffing even in the case of rice, and it has little or no effect in the case of other cereals such as corn. Other procedures have been tried without success, such as soaking the grains in a solution of salt with the object of modifying the grain proteins so as to aid subsequent puffing; coating the grains with moisture-impervious materials such as sugar, salt, albumen, or casein with the object of entrapping and retaining their moisture and thereby increasing the degree of puffing; case-hardening the grains with the same object in view; reducing the moisture content of the cooked cereal material to 5–14% just before puffing, together with reducing the grain to a plastic state by shredding or ribboning before such drying; etc. Despite all of these efforts, however, the only oven-puffed breakfast cereal on the market heretofore has been the rice product mentioned above.

One of the objects of the present invention is to provide new ready-to-eat, oven-puffed cereal foods having the toasted brown color, crispness, friability, and temporary imperviousness to moisture in milk or cream which characterize such products and render them highly appetizing.

Another object is to produce such products from cereals other than rice, as well as from rice itself.

A further object is to provide such products having a degree of puff greater than and crispness and friability superior to those obtainable heretofore even in the case of rice.

A still further object is to produce such superior products at minimum cost.

I have discovered that the waxy varieties of the cereal grains, such as corn, rice, sorghum, barley, millet, etc., oven-puff very easily and to a much higher degree of puff than the non-waxy varieties of the corresponding cereals. Thus puffed, toasted, crisp, moisture-impervious products can be produced by oven-puffing cereals which could not be satisfactorily treated by this method heretofore, and even in the case of rice a substantially improved and superior product is obtained. For example, waxy rice puffs two or three times as much as non-waxy rice when processed under identical conditions. Waxy sorghum and waxy corn puff three or more times as much as the non-waxy varieties of these cereals and more than non-waxy rice as heretofore marketed. Similar improvement in the degree of puffing is obtained in the case of waxy barley, etc.

The waxy varieties of the cereal grains are those in which the starch contained in the endosperm is stained red instead of blue with iodine, apparently because the starch has the molecular form known as amylopectin instead of that known as amylose. Other parts of the kernels of these waxy varieties may contain small amounts of non-waxy starch, but even though the whole grain is used so that all of this non-waxy starch is included in the puffed product, its proportion is insufficient to affect the results of the invention.

The whole grain may be processed in the form of whole kernels or fractions thereof such as grits,

which are hereinafter referred to together as grain elements, or the whole grain may be ground to flour and made into dough. On the other hand, the grain may be milled to eliminate most of the non-waxy starch which is found principally in a thin layer surrounding the endosperm beneath the bran and in the germ. The milled grain which consists practically entirely of waxy starch may then be processed as grain elements or ground into flour and made into a dough as in the case of the whole grain. Also purified starch obtained from the waxy cereals according to any known method of starch manufacture can be made into a dough and processed in essentially the same manner as dough made from flour. The expression "waxy cereal starch material" as used in the appended claims means any of the several types of starting materials mentioned above which consist predominantly of the waxy cereal starch in all cases, but may include small amounts of non-waxy starch, protein, etc.

The processing of the waxy material consists essentially of cooking and oven-puffing and may be carried out according to known practices of the art. Usually, grain elements are cooked in an autoclave or retort of the revolving type with added flavoring materials such as sugar, malt, salt, etc., whereas doughs are cooked in apparatus having special mixing means which provide sufficient heat transfer to permit the starch in the bulky mass of dough to be gelatinized. Cooking generally requires from 1½ to 2 hours when conducted at a temperature corresponding to 15 lbs. steam pressure, but in any case is continued until the starch is substantially gelatinized, and in the case of grain elements until the desired degree of caramelization and attendant color development has been effected.

The cooking period may be shortened by increasing the temperature or pressure, or by the addition of small amounts of ingredients such as citric acid, phosphoric acid, acid salts, and the like which alter the pH of the cook or otherwise accelerate the gelatinization of the starch or the caramelization. Moreover, it is usually desirable in the case of grain elements to cause them to imbibe water before cooking by pre-soaking them, preferably under pressure, in water which may contain such flavoring materials or accelerating agents as those aforementioned. These measures may be regulated so that if it is desired to maintain the grain elements in their integral form, cooking need not be so prolonged as to cause disintegration. After cooking, the moisture content in the case of grain elements is generally in the range of 30-40%, and the moisture content in the case of doughs is generally between 40 and 80%.

Cooked grain elements should be dried partially before puffing, either in the puffing apparatus or separately before being introduced therein, to a moisture content in the range of 5 to 25% and preferably between 7 and 15%. Also it is usually desirable to compress or otherwise form the grain elements, either before or after such partial drying, so as to facilitate puffing. For example, the elements may merely be flattened by passage through rolls or they may be shredded or extruded. Similarly cooked doughs should preferably be formed by shredding or extrusion into small pieces having at least one cross-sectional dimension that is sufficiently small to permit good heat transfer. However, partial drying of the dough pieces before puffing has less effect than in the case of grain elements and usually is not worthwhile.



Oven-puffing may be carried out in an inclined rotating perforated drum heated by an open flame or by hot gases and provided with internal ribs or fins which lift the material as the drum revolves and shower it through the heated atmosphere within the drum. Generally the temperature of the drum should be maintained within a range of 300-600° F. and it may be regulated so as to be substantially constant throughout the length of the drum or higher or lower at the inlet or outlet end of the drum as desired. The time required for the material to travel the length of the drum or in other words the period provided for puffing and toasting should be of the order of ½ to 3 minutes. It is desirable to effect the puffing and toasting simultaneously and if the time needed for puffing should be insufficient for toasting, additional saccharides can be used to facilitate caramelization. Other types of ovens such as the conduction type may also be used with a somewhat higher temperature range and a longer period for puffing and toasting.

The invention is illustrated by the following examples:

*Example 1*

35 lbs. of pearled waxy Cody sorghum, 10.1 lbs. of flavoring syrup, and 18.9 lbs. of water were cooked for 2 hours at 15 lbs. steam pressure, after which the cooked material was air-dried to 20% moisture and then tempered for a period of 20 hours. The tempered kernels were then passed through flaking rolls and oven-puffed at a temperature of 350-400° F. for about one minute by passage through a revolving drum. A puffed product of the desired degree of brown color, crispness and imperviousness to moisture was obtained, the puffed flakes having 6 to 12 times their original volume.

*Example 2*

Pearled waxy sorghum was ground into a flour capable of passing through an 80 mesh screen, and 15 lbs. of this flour were made into a dough with 13.75 lbs. of water containing 1.75 lbs. of sugar and 0.30 lb. of salt. This dough was transferred to a jacketed cooker equipped with suitable mixing means adapted for use in cooking doughs, and cooked for 30 minutes at the temperature corresponding to 20 lbs. steam pressure in the jacket. The cooked dough was extruded to ribbons of ⅜" x ⅛" and the ribbons were cut into pieces about ½" long which were oven-puffed at 550° F. for 5 minutes in a conductance oven. The resulting product comprised toasted, crisp pieces puffed to 3-4 times their original volume and having the desired degree of imperviousness to moisture in milk and cream.

*Example 3*

6 lbs. of waxy sorghum grain, 3.3 lbs. water, 0.7 lbs. sugar and 0.15 lb. of salt were cooked for 35 minutes in a pressure cooker at 25 lbs. steam pressure. The cooked grain was dried to about 7% moisture, ground to pass through 80 mesh and made into a dough of 40% moisture by admixing 6 lbs. of said flour with 3.5 lbs. of water. The dough was extruded, cut into dough pieces and oven-puffed as in Example 2 and a similar product was obtained.

*Example 4*

15 lbs. of pearled waxy maize, 1.8 lbs. of sugar, 0.43 lb. of salt and 8.45 lbs. of water were cooked

for 2 hours at 15 lbs. steam pressure, after which the cooked material was air-dried to 18% moisture and then tempered for 20 hours. The tempered kernels were then passed through flaking rolls and oven-puffed at a temperature of 350–400° F. for about one minute by passage through a toasting oven (perforated cylinder). A puffed product of the desired degree of brown color, crispness and imperviousness to moisture was obtained, the flakes having puffed two to three times as much as flakes made from non-waxy corn or maize and processed under identical conditions.

*Example 5*

15 lbs. of California waxy rice, 1.31 lbs. of sugar, 0.44 lb. of salt and 5 lbs. of water were cooked for 1⅔ hours at 15 lbs. steam pressure, after which the cooked material was dried to 18–20% moisture and tempered for 20 hours. The tempered kernels were then passed through flaking rolls and oven-puffed at a temperature of 350–400° F. for about one minute by passage through a toasting oven (perforated cylinder). A puffed product of the desired degree of brown color, crispness and imperviousness to moisture was obtained, the flakes having puffed two to three times as much as flakes made from non-waxy varieties of rice and processed under identical conditions.

*Example 6*

Pearled waxy barley was cooked with water, sugar and salt for 1½ hours at 13 lbs. steam pressure in an autoclave. The cooked material contained about 35% moisture and was dried to 19.4% moisture and tempered for 12 hours. The tempered kernels were then passed through flaking rolls and oven-puffed under conditions similar to those set forth in the preceding examples. A puffed product of the desired color and crispness was obtained, the flakes having puffed approximately twice as much as flakes made from non-waxy barley and processed under the same conditions.

*Example 7*

5.8 lbs. of purified waxy sorghum starch were mixed with 3.5 lbs. of water, 0.7 lbs. of sugar and 0.15 lb. of starch, and the mix was then cooked in a jacketed dough mixer for 20 minutes at the temperature corresponding to 20 lbs. steam pressure in the jacket. The cooked material was extruded in shreds which were allowed to dry at room temperature to eliminate surface stickiness. The shreds (about 30% moisture) were then cut into pieces about ½" long which were oven-puffed at 550° F. for 5 minutes in a conductance oven. The resulting product comprised toasted, crisp pieces puffed to approximately 5 times their original volume and more than twice as much as similar pieces made from non-waxy starch and processed under the same conditions.

As noted above the use of whole grain results in the presence of small amounts of non-waxy starch in the product. It will also be understood that commercial crops even when grown from 100% waxy seed may nevertheless include some non-waxy grains due to cross pollination or other causes. Care in the supervision of growing conditions and in the selection of crops will keep the resultant proportion of non-waxy starch so low that it does not substantially affect the results of the invention when the product is made from a flour or dough. However, when the mixed grain is processed as grain elements, some of the puffed pieces in the final product may come entirely from non-waxy kernels. Such pieces will not puff to the same extent as those derived from waxy kernels and hence they may be removed by screening if their number should be excessive.

The invention is not restricted to the examples set forth above and it will be evident to those skilled in the art that various changes can be made in the details of procedure without departing from its spirit. Reference should therefore be had to the appended claims for a definition of the limits of the invention.

What is claimed is:

1. A process for preparing a puffed cereal food product which comprises the steps of cooking waxy cereal starch material and oven-puffing the cooked material.

2. A process for preparing a puffed cereal food product which comprises the steps of cooking a waxy cereal starch material and subjecting small masses of the cooked material to an elevated temperature in the range of 300° F. to 600° F. at atmospheric pressure to toast and puff the product.

3. A process for preparing a puffed cereal food product which comprises the steps of cooking a waxy sorghum starch material and oven-puffing the cooked material.

4. A process for preparing a puffed cereal food product which comprises the steps of cooking a waxy maize starch material and oven-puffing the cooked material.

5. A process for preparing a puffed cereal food product which comprises the steps of cooking a waxy rice starch material and oven-puffing the cooked material.

6. A cereal food product comprising crisp, friable, toasted and puffed particles of cooked waxy cereal starch material.

7. A cereal food product comprising crisp, friable, toasted and puffed particles of cooked waxy sorghum starch material.

8. A cereal food product comprising crisp, friable, toasted and puffed particles of cooked waxy maize starch material.

9. A cereal food product comprising crisp, friable, toasted and puffed particles of cooked waxy rice starch material.

MATTISON WELLS ALDERMAN.

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# MGG

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Volume 248 Number 3 21 August 1995


## Original papers



**Continuation see back cover page**

Mol Gen Genet (1995) 248: 253-259



ORIGINAL PAPER

Toshiki Nakamura · Makoto Yamamori
Hisashi Hirano · Soh Hidaka · Tukasa Nagamine

# Production of waxy (amylose-free) wheats

Received: 13 October 1994 Accepted: 3 January 1995

**Abstract** The Waxy (Wx) protein has been identified as granule-bound starch synthase (GBSS: EC 24.1.21), which is involved in amylose synthesis in plants. Although common wheat (*Triticum aestivum* L.) has three Wx proteins, "partial waxy mutants" lacking one or two of the three proteins have been found. Using such partial waxy mutants, tetra- and hexaploid waxy mutants with endosperms that are stained red-brown by iodine were produced. Both mutants showed loss of Wx protein and amylose. This is the first demonstration of genetic modification of wheat starch.

**Key words** Wheat · Waxy · Amylose · Wx protein · Mutant

## Introduction

Recently, many genes encoding enzymes involved in starch synthesis have been cloned and used for genetic manipulation (Visser and Jacobsen 1993; Smith and Martin 1993; Müller-Röber and Koßmann 1994). One of these enzymes, granule-bound starch synthase (GBSS: EC 24.1.21), is the key enzyme in amylose synthesis (Preiss 1991). GBSS binds tightly to starch granules and is known as Wx protein (Echt and Schwartz 1981; Vos-Scheperkeuter et al. 1986; Preiss 1991). Amylose and amylopectin are the two major polysaccharide components of reserve starch in plants. The amylose content in total reserve starch varies from 11%–37% in nonmutant phenotypes (Shannon and Garwood 1984), whereas "waxy" (amylose free or glutinous) mutants have essentially no amylose. Waxy mutants also lack Wx protein, with the exception of several maize waxy mutants (Echt and Schwarz 1981). Nonwaxy and waxy phenotypes are easily distinguishable by staining of starch with iodine. Nonwaxy starch stains blue-black while waxy starch stains red-brown.

Waxy mutants have been identified in cereals such as maize, rice, barley, sorghum and amaranth. However, waxy mutants have not been reported in tetra- or hexaploid wheat. Common wheat has three homoeologous *waxy* genes, *WX-A1*, *WX-B1* and *WX-D1*, located on the group 7 chromosomes (Chao et al. 1989). It was thought likely that these genes produce at least three GBSS isozymes, as found for many wheat homoeologous gene-encoded functional proteins (Hart 1987). Indeed a modified two-dimensional gel electrophoresis (2D-PAGE) method (Nakamura et al. 1993a) enabled us to identify three Wx proteins, Wx-A1, Wx-B1 and Wx-D, which have slightly different molecular weights and or isoelectric points (Fig. 1). Using this technique, we were able to identify mutants (Nakamura et al. 1993b, c; Yamamori et al. 1994), each lacking one or two Wx proteins, which were termed "partial waxy mutants". Wild type was designated Type 1. Type 2 is lacking in Wx-A1 protein, Type 3 in Wx-B1, Type 4 in Wx-D1 and Type 7 in both Wx-A1 and Wx-B1 (Fig. 2). In this study, we describe the production of waxy tetra- and hexaploid wheats through the use of these mutants. Since wheat is one of the most widely cultivated crops in the world and its starch is used in food and nonfood industries, waxy wheats will have important implications in both fields.

...mmunicated by H. Saedler

...akamura (✉) · S. Hidaka
...oku National Agricultural Experiment Station,
...ahira 4, Morioka, Iwate 020-01, Japan

... Yamamori · T. Nagamine
...pan International Research Center for Agricultural Sciences,
...kinawa Branch, Ishigaki, Okinawa 907, Japan

... Hirano
...ational Institute of Agrobiological Resources, Kannondai 2-1-2,
...ukuba, Ibaraki 305, Japan

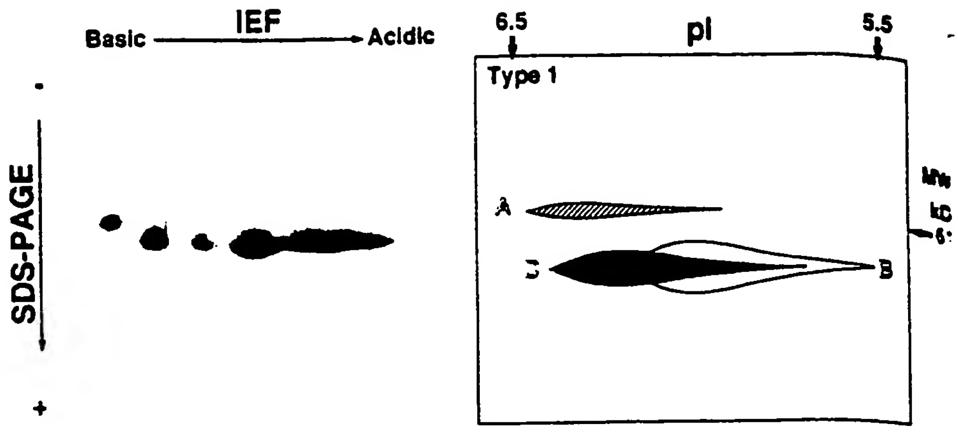


**Fig. 1** Two-dimensional gel electrophoresis pattern of Wx protein of common wheat (*Triticum aestivum* L.) (left panel) and a diagrammatic representation of the pattern (right panel). The diagrams show differences in molecular weight (*MW*), isoelectric point (*pI*), and amount (size of the torpedo shapes) of three Wx proteins. *A*, *B* and *D* in the diagram indicate the three Wx proteins Wx-A1, Wx-B1 and Wx-D1, respectively. The normal phenotype has the three Wx proteins and is designated Type 1

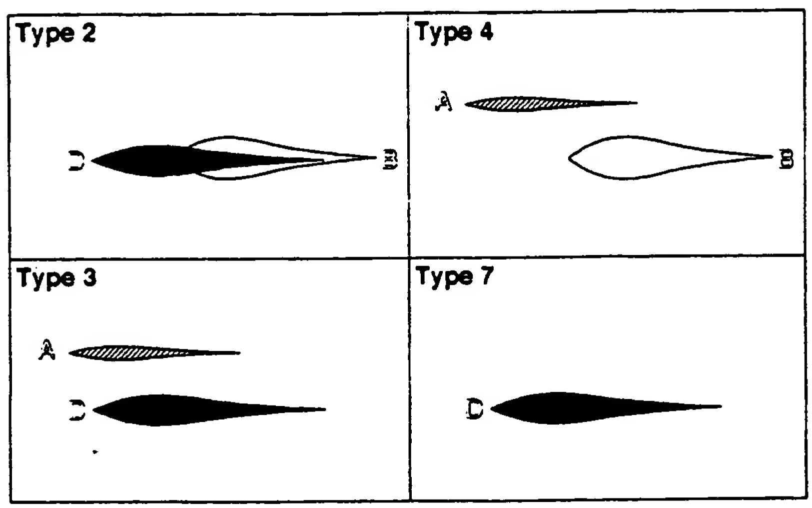


**Fig. 2** Schematic diagrams of Wx protein patterns in partial waxy mutants. The Type 2 mutant lacks Wx-A1 protein. Type 3 lacks Wx-B1 protein. Type 4 lacks Wx-D1 protein, and Type 7 lacks both Wx-A1 and Wx-B1 proteins

## Materials and methods

### Plant materials

Two common wheats, Kanto 107 (K107) and Bai Huo, and a durum wheat (*Triticum durum* Desf.) cv. Aldura were used. K107 is a Type 7 mutant and Bai Huo is a Type 4 mutant (Yamamori et al. 1994; Fig. 2). Aldura has both Wx-A1 and Wx-B1 proteins. Embryos of the $F_2$ seeds of K107 × Aldura, in which endosperms stained red-brown with iodine, were sterilized, excised and germinated on MS medium without hormone. At the three-leaf stage, the plants were transplanted into horticultural soil (Kureha) and grown in a greenhouse at 21° C(day) 15° C(night).

### Starch granule preparation

Starch granules were prepared as described previously (Nakamura et al. 1993a). The starch granules were purified using SDS buffer containing 60 mM TRIS-HCL pH 6.8, 3% (w/v) SDS, 3% (v/v) β-mercaptoethanol and 10% (v/v) glycerol. All the steps in the preparation were performed at 4° C. Purified starch granules were stored at −20° C.

### Extraction of Wx protein and electrophoresis

Protein extraction for SDS-PAGE was performed as described by Echt and Schwartz (1981). For 2D-PAGE, 10 mg of the prepared starch granules were mixed with 300 µl of lysis buffer [8 M urea, 2% (v/v) Nonidet-P40, 2% (v/v) Ampholine pH 3.5 ~ 10, 5% (v/v) β-mercaptoethanol and 5% (w/v) polyvinylpyrrolidone] and heated in a boiling water bath for 2 min, then cooled on ice. SDS-PAGE was performed by the system of Laemmli (1970) using 10% acrylamide gels and 2D-PAGE was performed as previously reported (Nakamura et al. 1993a). After electrophoresis, gels were stained with a silver staining kit (Wako Pure Chemicals) or Coomassie Brilliant Blue R.

### Iodine staining

One month after anthesis, fresh seeds were harvested, endosperms were cut into halves, and one-half was stained by iodine solution (740 mg resublimed iodine and 1480 mg potassium iodide dissolved in 400 ml deionized distilled water). Starch granules were also stained with this solution and analyzed microscopically.

**Table 2** Segregation of waxy type seeds in $F_2$ seeds of K107 × Bai Huo

| $F_1$ plant | Number of seeds | KI-$I_2$ staining of endosperms | | $\chi^2$ (63:1) | P |
|---|---|---|---|---|---|
| | | Blue-black | Red-brown | | |
| No. 1 | 556 | 549 | 7 | 0.33 | 0.50–0.80 |
| No. 2 | 728 | 719 | 9 | 0.52 | 0.30–0.50 |
| No. 3 | 511 | 504 | 7 | 0.12 | 0.50–0.80 |
| Total | 1795 | 1772 | 23 | | |


**Fig. 4** Endosperms stained with iodine solution. The $F_2$ seeds K107 (Type 7) × Bai Huo (Type 4) included segregants with red-brown staining endosperm (waxy type)

Four seeds in which endosperms and starch granules stained red-brown by iodine segregated from 288 $F_2$ seeds matured on the $F_1$ plants (Table 1). There was no Wx protein in the starch granules of these four seeds. The nonwaxy $F_2$ seeds from this cross all had Wx protein, although the protein level varied (Fig. 3).

Chromosome numbers in the four $F_2$ plants were decreased from the 35 of the $F_1$ pentaploid to 29, 29, 30 and 29, respectively (Table 1). All $F_3$ seeds matured on the four $F_2$ plants showed the waxy phenotype and three randomly chosen $F_3$ plants all had a chromosome number of 28 (Table 1). N- and C-banding of chromosomes indicated that the D genome was absent in these plants (data not shown). The waxy phenotype and chromosome number of $F_3$ individuals was stably transmitted to the $F_4$ generation (Table 1).

The level of amylose (0.6%–0.7% of starch) in $F_3$ and $F_4$ seeds was comparable to that in a waxy maize (0.6%), although both parents had amylose contents greater than 25% (Table 1). Seed starch was analyzed by gel filtration using a Sephacryl S-1000 column. Only an amylopectin peak was seen in the mutant, whereas both parents showed amylopectin and amylose peaks (data not shown).

## Type 7 × Type 4

Waxy hexaploid wheat could be produced by crossing Type 7 with Type 4 mutants. Based on Mendelian segregation, the $F_2$ generation should include individuals lacking all three Wx proteins. These plants should be waxy mutants, because mutants lacking Wx protein in various diploid plants are all waxy mutants (Echt and Schwartz 1981; Sano 1984; Konishi et al. 1985; Hovenkamp-Hermelink et al. 1987).

All $F_2$ seeds matured on the three $F_1$ plants of K107 × Bai Huo were harvested and endosperms were stained with iodine solution. Twenty-three seeds staining red-brown were identified among 1795 $F_2$ seeds

Varagona 1985; Okagaki and Wessler 1988). In maize, large insertions (150 bp ~ 6.1 kbp) or deletions (> 300 bp) are associated with mutations among the *wx* alleles, while single base changes or very small insertions or deletions were responsible for the mutations in rice. The mechanism of origin of the spontaneous lesions detected in wheat *wx* alleles appears to differ among the three *waxy* loci, and to be more complicated than in maize and rice.

Starch is a major component of the harvested parts of plants and is used in food and non-food industries. Demand for many kinds of natural starch that possess novel physical and chemical properties is increasing, due to the desire to develop new starch products and to reduce the need for post-harvest chemical modification processes (Smith and Martin 1993). A number of naturally occurring mutants with altered proportions of amylose and amylopectin have been well studied (Shannon and Garwood 1984). Of these, waxy maize is widely used in food industries. Waxy maize starch is notable for paste clarity, high water binding capacity and resistance to gel formation and retrogradation (Watson 1988). Starch of amylose-free potatoes yields a similar type of clear paste, which does not retrograde, and seems to be useful in ready-prepared foods (Visser and Jacobsen 1993). Starch of waxy wheats also lacks amylose and may have several applications, including reduction of staling in flour products, especially bread.

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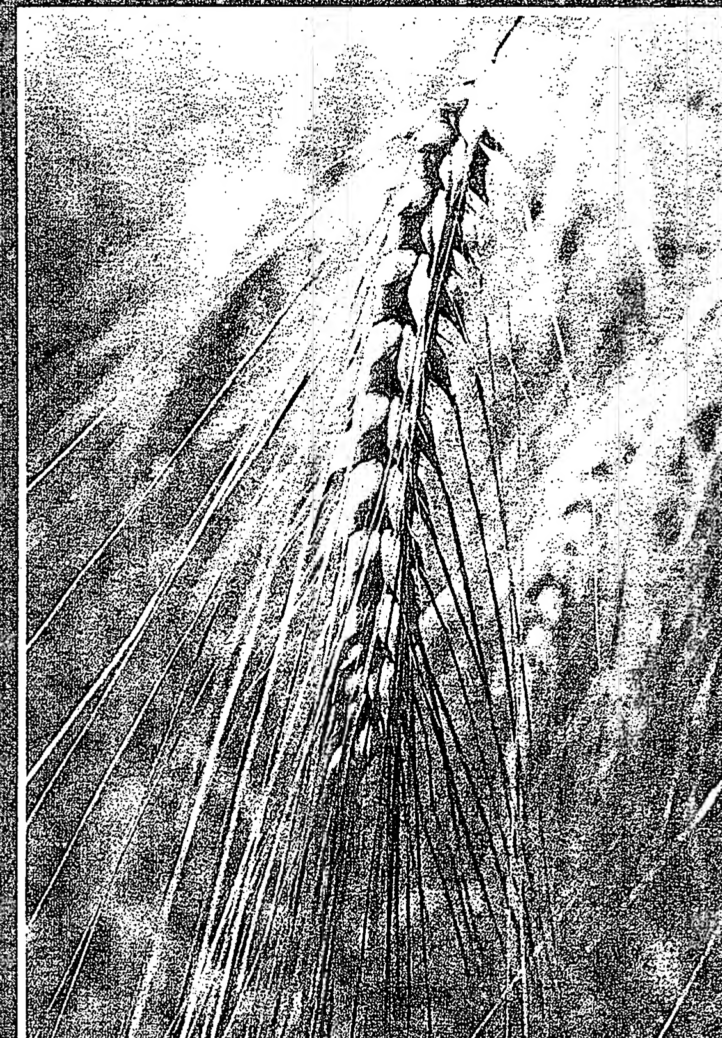
# WHEAT
## Chemistry and Technology



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3340 Pilot Knob Road
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cereals in their unique viscoelastic properties. This property, conferred on bread wheat by the introduction of the D genome, is responsible for the universal use of wheat for a wide range of products. Among these products are pan bread, noodles, cakes, biscuits, cookies, steamed bread, doughnuts, croissants, bagels, flat breads, and chapati. Each of these products is ideally produced from a wheat selected to provide flour with the required characteristics. Moss (1973) summarized the requirements for the balance of grain hardness and protein content for several common products (Fig. 1).

One application of wheat flour that is growing in importance is its separation into starch (carbohydrate) and gluten (protein). These two coproducts each have applications in food and industrial products, but it is the unique physical properties of gluten that make the process economically viable. The separation of starch and gluten is achieved in various ways but basically involves hydration of the flour to form a dough or batter and then a combination of washing, screening, and centrifugation.

World production of gluten and starch increased greatly in the past decade (Sosland, 1986). In 1980, gluten production was estimated at 80,000 tonnes; by 1986, it had grown to 253,000 tonnes. The major increase was in Western Europe, where production tripled between 1980 and 1986 and a further doubling

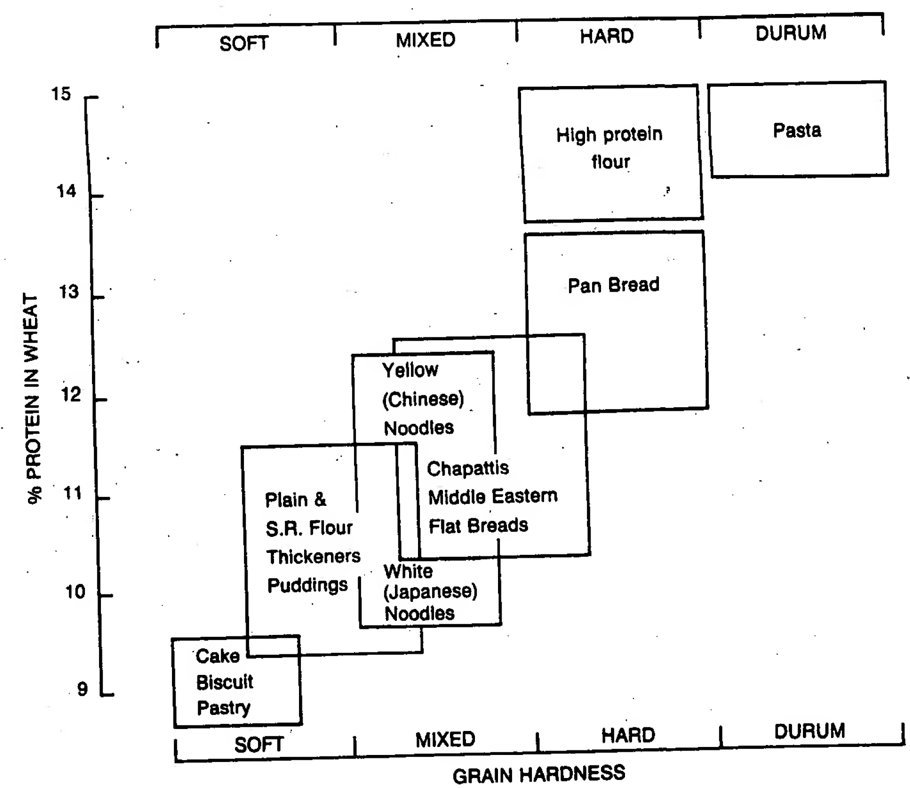


Fig. 1. Wheat types for various end products. S.R. = self raising. (Adapted from Moss, 1973)

combination, for many varied breads.

Descriptive data such as break flour yields, alkaline water retention, absorption, and mixograph characteristics are used to describe both soft white wheat and soft red wheat, and these two types of wheat are used for many of the same purposes. In discussing wheat quality, it is best to first look at the different kinds or types of wheat.

## II. BOTANICAL CHARACTERISTICS THAT DETERMINE WHEAT QUALITY

### A. Species

Three significant species of wheat are grown in North America: *Triticum aestivum* L., to which most common varieties belong; *T. compactum* Host, to which the club wheats belong; and *T. durum* Desf., to which the durum wheats belong. The grain of these three species differs considerably in quality characteristics, and the differences are reflected in the uses made of the milled products.

The classes hard red winter, hard red spring, soft red winter, and soft and hard white belong in the species *T. aestivum*. The class of white wheat has subclasses in two species, soft and hard white winter in *T. aestivum* and club wheat in *T. compactum*. The classes durum and red durum wheat belong in the species *T. durum*.

A wide range of wheat quality characteristics is available in *T. aestivum* (in fact, the complete range of quality available from U.S. wheat), with the exception of club and durum wheats. Kernel hardness and protein content vary widely within this species. Common wheat may be of either winter or spring growing habit and may have either red or white kernels. An outstanding characteristic of this species, from the standpoint of economic value, is that its flour is superior to that of all other species for the production of leavened bread. Although most common wheat is used for bread flour production, varieties have also been developed that are particularly suited to the production of cake, cookie, biscuit, cracker, and pastry flours. Climatic and soil conditions also have a marked influence on the suitability of common wheat for specific types of flour.

Club wheat (*T. compactum*) accounts for a minor portion of the U.S. wheat crop and is produced principally in Washington, Oregon, Idaho, and California. Very little club wheat is produced in Canada. Both winter and spring varieties are grown. Although there are varieties of club wheat with red kernels, commercial production in the United States is confined to the white varieties. The kernels have a soft texture and low protein content. Club wheat is not well suited for bread flour but is excellent for certain types of cake and pastry flours, where low protein content and weaker glutens are desired.

Durum wheat (*T. durum*) is produced mainly in the north central United States (especially North Dakota) and the southwestern United States, in Saskatchewan in Canada, and in the adjoining states and provinces. Durum wheat has a spring growing habit. Varieties with red kernels are grown to a very limited extent for feed purposes, but the principal production is of the amber (white) varieties. The kernels are generally very hard in texture and rather high in

protein content. Most of the durum wheat crop is used for the production of semolina or durum flour for pasta products because the qualities of durum gluten make it particularly desirable for this use but rather less suitable for breadmaking.

### B. Varieties

A further step in the biological classification of wheat is the variety. New varieties are usually bred with a specific purpose in mind, such as disease resistance, yield performance, or a set of quality characteristics. Even within a variety, some quality characteristics may vary widely because of environmental effects.

Varieties in *T. aestivum* range in quality characteristics from varieties suitable for the production of high-gluten flour on one end to those suitable for angel food cake flour on the other end. The low-protein wheats of *T. compactum* varieties are used primarily in cake and cookie production, and *T. durum* varieties provide a substantial portion of the wheat used to produce flour or semolina for pasta. Varietal differences in grain quality are great among the common wheats, less so among the durum wheats, and relatively small among the club wheats.

In recent years, increasing emphasis has been placed on the quality of grain for processing, specifically the milling quality and the baking or (in the case of durum wheat) pasta-making quality. Wheat varieties that produce grain of inferior processing value are being eliminated as rapidly as possible. The production of such varieties usually stops as soon as varieties with equally satisfactory agronomic properties and superior processing quality become available. About 200 recognized varieties of wheat are produced commercially in the United States, although about 50 varieties make up the bulk of the crop (USDA-FGIS, 1984).

Although variety is an important factor influencing wheat quality, wheat is seldom marketed on the basis of individual variety. It is common practice to segregate wheat as it comes to the market according to class, each class consisting of a group of varieties with somewhat similar characteristics and generally suited for similar purposes.

## III. PHYSICAL CHARACTERISTICS THAT DETERMINE WHEAT QUALITY

As stated previously, wheat quality has a different meaning or definition for each intended use of the grain. Good-quality wheat for making breads and specialty rolls must have a strong, extensible gluten, whereas good-quality wheat for baking cakes or cookies has only minimal gluten. It is highly unlikely that any one wheat could satisfy both needs. Thus, physical tests and observations of wheat actually describe some of its characteristics rather than evaluate its quality.

In the process of marketing, wheat is often assigned a numerical grade, which depends on the results of certain tests. These tests include test weight, percent of damaged kernels, and the presence of material other than the predominant grain (foreign material, dockage, etc.).